CLAIMS

1. The use of an effective amount of one or more catalysts which is/are enzyme(s) belonging to the heme biosynthetic pathway, or an enzymatically equivalent part or analogue thereof, together with a pharmaceutically acceptable carrier, for the preparation of a pharmaceutical composition for treatment or prophylaxis of a disease caused by a deficiency, in a subject, of an enzyme belonging to the heme biosynthetic pathway.

2. The use according to claim 1, wherein the disease is selected from the group consisting of
acute intermittent porphyria (AIP),
ALA deficiency porphyria (ADP),
Porphyria cutanea tarda (PCT),
Hereditary coproporphyria (HCP),
Harderoporphyria (HDP),
Variegata porphyria (VP),
Congenital erythropoetic porphyria (CEP),
Erythropoietic protoporphyria (EPP), and
Hepatoerythropoietic porphyria (HEP).

3. The use according to claim 1, wherein the catalyst is one or more enzymes selected from the group consisting of
delta-aminolevulininic acid synthetase,
delta-aminolevulinic acid dehydratase (ALAD),
porphobilinogen deaminase (PBGD),
uroporphyrinogen III cosythetase,
uroporphyrinogen decarboxylase,
coproporphyrinogen oxidase,
protoporphyrinogen oxidase, and
ferrochelatase,
or an enzymatically equivalent part or analogue thereof.

4. The use according to claim 1, wherein the disease is AIP and the enzyme is PBGD or an enzymatically equivalent part or analogue thereof, preferably in combination with ALAD.

5. The use according to claim 1, wherein the catalyst is a recombinant form of the enzyme belonging to the heme biosynthetic pathway or of the enzymatically equivalent part or analogue thereof.

6. The use according to any of the preceding claims, wherein the pharmaceutical composition is prepared to be administered by a route selected from the group consisting of the intravenous route, the intraarterial route, the intracutaneous route, the subcutaneous route, the oral route, the buccal route, the intramuscular route, the anal route, the transdermic route, the intradermal route, and the intratechal route.

7. The use according to any of the preceding claims, wherein the catalyst is formulated in an isotonic solution, such as 0.9% NaCl and 10-50 mM Sodium phosphate pH 6.50 to 8 or Sodium phosphate, glycine, mannitol or the corresponding potassium salts.

8. The use according to claim 7, wherein the catalyst is lyophilised.

9. The use according to claim 8, wherein the catalyst is sterile filtered.

10. The use according to any of the preceding claims, wherein the catalyst is formulated as lipid vesicles comprising phosphatidylcholine or phosphatidylethanolamine or combinations thereof.

11. The use according to any of the preceding claims, wherein the catalyst is incorporated into erythrocyte ghosts.

12. The use according to any of the preceding claims, wherein the catalyst is formulated as a sustained release formulation involving biodegradable microspheres, such as microspheres comprising polylactic acid, polyglycolic acid or mixtures of these.

13. The use according to any of the preceding claims, wherein the catalyst is lyophilised in a two-compartment cartridge, where the catalyst will be in the front compartment and water for reconstitution in the rear compartment.

14. The use according to claim 13, wherein the two compartment cartridge is combined with an injection device to administer the catalyst either by a needle or by a needle-less (high pressure) device.

15. The use according to any of the preceding claims, wherein the catalyst is formulated in a physiological buffer containing an enhancer for nasal administration.

16. The use according to claim 1, wherein the catalyst is formulated as an oral formulation containing lipid vesicles, such as those comprising phospatidylcholine, phosphatidylethanolamine, or sphingomyeline, or dextrane microspheres.

17. The use according to claim 1, wherein the catalyst is formulated so as to enhance the half-life thereof in the subject's bloodstream.

18. The use according to claim 17, wherein the catalyst has a polyethylene glycol coating.

19. The use according to claim 17, wherein the catalyst is complexed with a heavy metal.

20. The use according to claim 1, wherein the catalyst is an enzymatically equivalent part or analogue of the enzyme and exerts at least part of its enzymatic activity intracellularly upon administration to the subject.

21. The use according to claim 20, wherein the catalyst is a small artificial enzyme or an organic catalyst which can polymerise porphobilinogen to hydroxymethylbilane

22. The use according to claim 1, wherein the catalyst is said enzyme formulated in such a manner that it exerts at least part of its enzymatic activity intracellularly upon administration to the subject.

23. The use according to claim 22, wherein the catalyst is tagged with specific carbohydrates or other liver cell specific structures for specific liver uptake.

24. The use according to claim 1, wherein the catalyst exerts substantially all its enzymatic activity extracellularly in the bloodstream.

25. The use according to claim 24, wherein the enzymatic activity of the catalyst on its relevant heme precursor results in a metabolic product which 1) either moves into the intracellular compartment and is converted further via the remaining steps of the heme biosynthetic pathway or 2) is excreted from the subject via urine and/or faeces.

26. The use according to claim 1, wherein the catalyst has been prepared by a method comprising

a) introducing, into a suitable vector, a nucleic acid fragment which includes a nucleic acid sequence encoding the catalyst;
b) transforming a compatible host cell with the vector;
c) culturing the transformed host cell under conditions facilitating expression of the nucleic acid sequence; and
d) recovering the expression product from the culture
and optionally subjecting the expression product to post-translational processing, such as in vitro protein refolding, enzymatic removal of fusion partners, alkylation of amino acid residues, and deglycosylation, so as to obtain the catalyst.

27. The use according to claim 1, wherein the catalyst has been prepared by liquid-phase or solid-phase peptide synthesis.

28. The use according to claim 26 of the preceding claims, wherein the catalyst is free from any other biological material of human origin.

29. The use according to any claim 1, wherein the catalyst is to be administered at least once a day, such as 2, 3, 4, or 5 times daily.

30. The use according to claim 29 wherein the daily dosage is in the range of 0.01 – 1.0 mg/kg body weight per day, such as in the range of 0.05 – 0.5 mg/kg body weight per day.

31. The use according to claim 29, wherein the daily dosage is about 0.1 mg per kg body weight per day.

32. The use according to any of the preceding claims wherein the catalyst is a recombinant form of the enzyme.

33. The use according to claim 32 wherein the catalyst is recombinant human PBGD based on any of Seq. ID NO 3 (clone PBGD 1.1) and Seq. ID NO 4 (non-erythro PBGD 1.1.1).

34. The use of a human PBGD cDNA sequence of either non-erythropoietic form or erythropoietic form to prepare, either alone or in a combination with a suitable genetic vector and other components, a composition that can be used for gene therapy of a patient having a mutation in the PBGD gene causing an enzyme defect.

35. The use according to claim 34, wherein the enzyme defiency is selected from enzyme deficiencies resulting in a disease selected from the group of Acute Intermittent Porphyria, (AIP), ALA deficiency porphyria (ADP), Porphyria cutanea tarda (PCT), Hereditary coproporphyria (HCP), Harderoporphyria (HDP), Variegata porphyria (VP), Congenital erythropoietic porphyria (CEP), Erythropoietic protoporphyria (EPP), and Hepatoerythropoietic porphyria (HEP).

36. The use according to claim 35 wherein the disease is Acute Intermittent Porphyria, (AIP).

37. The use according to claims 34-36, wherein the human PBGD cDNA sequence is selected from the group of Seq. ID NO 3 (clone PBGD 1.1) or Seq. ID NO 4 (non-erythro PBGD 1.1.1).

38. The use according to any of claims 34-37 wherein the said genetic vector is selected from the group of plasmids, adenovirus, retrovirus or associated adenovirus.

39. The use according to claims 34-38 wherein the application of said formulation results in substantially normal PBGD activity measured as a normalisation in urinary and/or serum levels of delta-aminolevulinic acid (ALA) and porphobilonogen (PBG) compared to the levels before treatment or to a reduction in the frequency of attack of symptons.

40. The use of a human PBGD cDNA sequence of either non-erythropoietic form or erythropoietic form to prepare either alone or in a combination with a suitable genetic vector and other components a composition facilitating the treatment of a patient with

Acute Intermittent Porphyria (AIP) by correction of one of the specific point mutations identified causing AIP by gene therapy induced chimeraplasty gene repair.

41. The use according to claim 40 comprising a delivery system for transfection which is based on the use of a non-viral vector formulated in a vehicle preparation comprising one or more components selected from cationic phospholipids, phospholipids, phospholipids mixed with neutral lipids, lictosylated PEI, liposomes liposomes comprising mixtures of natural phopholipids and neutral lipids.

42. A use according to claim 40 wherein the mutation is selected from Table A.

43. A catalyst which is an enzyme of the heme biosynthetic pathway selected from the group consisting of
delta-aminolevulininic acid synthetase,
delta-aminolevulinic acid dehydratase (ALAD),
porphobilinogen deaminase (PBGD),
uroporphyrinogen III cosythetase,
uroporphyrinogen decarboxylase,
coproporphyrinogen oxidase,
protoporphyrinogen oxidase, and
ferrochelatase,
or an enzymatically equivalent part or analogue thereof, for use as a medicament.

44. A catalyst according to claim 43, which is recombinant human PBGD based on any of Seq. ID NO 3 (clone PBGD 1.1) and Seq. ID NO 4 (non-erythro PBGD 1.1.1).

45. An expression plasmid pExp1-M2-BB as shown in Seq. ID NO 1 for use in the expression of rhPBGD in E. coli.

46. A DNA fragment, EcoR I - Hind III linear fragment as shown in Seq. ID NO 2, capable of obtaining hemC-deletion in a host.

47. A production strain of rhPBGD obtained by use of the DNA fragment, EcoR I - Hind III linear fragment as shown in Seq. ID NO 2 to obtain hemC-deletion in the host JM105-H-R6-C by homologous gene replacement and transforming the resulting strain with the

expression plasmid pExp1-M2-BB to yield the final production strain PBGD which is free from production of PBGD of non human origin (Accession No 12915).

48. A method for the preparation of rhPBGD by a method comprising

a) introducing, into a suitable vector, a nucleic acid fragment which includes a nucleic acid sequence encoding PBGD;
b) transforming a compatible host cell with the vector;
c) culturing the transformed host cell under conditions facilitating expression of the nucleic acid sequence;
d) recovering the expression product from the culture.

49. A method according to claim 48 further comprising a fermentation step.

50. A method according to claim 48 further comprising a purification step.

51. A method according to claim 50 wherein the purification is performed wiht a His-Tag (rhPBGD-His).

52. A rhPBGD having a stability of at least 6 weeks at 20°C, such as for at least 7 weeks, preferably for 8 weeks.

53. A rhPBGD having a stability resulting in a decrease in activity of less that 10% per month, such as less than 5%.